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ASSESSMENT OF THE INFLUENCE OF NATRIUM CHLORIDE WATER ON THE COURSE OF TOXIC LIVER DAMAGE IN THE EXPERIMENT

Gushcha S.G.[1]; Kucherenko M.P.[2]*; Nasibullin B.A.[1]; Strus O.E.[3]; Volyanska V.S.[4]; Novikova A.I.[5]; Kovalchuk L.I.[2]; Romanenko I.Y.[6]; Romanenko I.V.[7]

[1]State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", Odesa, Ukraine
[2]State Institution "South Ukrainian National Pedagogical University named after K. D. Ushynsky", Odesa, Ukraine
[3]Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[4]Odessa National Medical University, Odesa, Ukraine
[5]Odesa I.I. Mechnikov National University, Ukraine
[6]Ukrainian Scientific and Practical Center for Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine, Kyiv, Ukraine
[7]Lugansk State Medical University, Rubizhne, Ukraine

*docnickolask@gmail.com

Abstract

The paper presents data on the evaluation of the effectiveness of the use of natural chloride natrium mineralized water (MW) on the condition of the body of rats with alcohol intoxication (AI). Alcoholization lasted 30 days. From 15 to 30 days, the animals received MW. The use of MW in rats on the background of the development of AI had a positive effect on the state of metabolism: the processes of reamination, protein metabolism, energy-dependent transmembrane transport were restored, there was a decrease in the manifestations of hypoxic nature; the activity of endogenous detoxification processes increased and the state in the system of lipid peroxidation and antioxidant protection was restored. Microscopically, the liver parenchyma has improved (lipid and hyaline inclusions disappear, blood circulation increases, dystrophy is reduced). The obtained data show that the use of sodium chloride MW has a protective effect on the structural and functional state and metabolic parameters of the rats liver.

Keywords: alcohol intoxication, structural and functional state of the liver, natrium chloride mineral water.

Introduction

To date, diseases associated with the toxic effects of alcohol, occupy a significant place among the pathologies, as they cause disturbances in the functioning of all systems of the human body [1, 2, 3]. But the liver suffers the most from the action of alcohol, as it takes the first and the main blow from the toxic effects of alcohol and toxic products of its metabolism (aldehyde acetate) [4]. Alcohol is metabolized primarily in hepatocytes, which make up about 70 percent of the liver's mass. Hepatocytes express very high levels of the main enzymes that oxidize ethanol, alcohol dehydrogenase (ADH), which is located in the cytosol, and cytochrome P450 2E1 (CYP2E1), which is located in the smooth endoplasmic reticulum [5]. Hepatocytes also express high levels of catalase (the second pathway of ethanol oxidation). This enzyme is found in peroxisomes. Catalase usually detoxifies hydrogen peroxide (H_2O_2) to water and oxygen [6]. Ethanol oxidation can result from a combination of reactions catalyzed by NADPH oxidase (microsomal), xanthine oxidase, and catalase. Although the role of catalase in ethanol metabolism is not fully understood, peroxidation appears to be the most important pathway for ethanol metabolism [7]. A third metabolic pathway of ethanol oxidation has also been described that occurs in the endoplasmic reticulum of the hepatocyte and is called the "microsomal ethanol oxidation system" (MEOS) [7, 8]. It uses the terms of the enzymatic system "drug metabolism" (a mixed function oxidase system driven by a microsomal electron transport chain). MEOS with NADPH oxidase as a cofactor also oxidizes ethanol to acetaldehyde.

Liver damage occurs as a result of the following major pathological mechanisms [9, 10]:

- liver cells begin to use alcohol as a substrate for energy metabolism, while stopping the oxidation of fatty acids, which are a source of energy in the norm, resulting in their accumulation in the cell, i.e. develops fatty liver disease;
- the function of many liver enzyme systems is disturbed (aspartate aminotransferase, alanine aminotransferase, alcohol dehydrogenase, etc.);
- alcohol stimulates the collagen production, which leads to fibrosis, which is primarily observed in the portal tract and / or terminal venules;
- neutralization of alcohol leads to significant consumption of oxygen in the liver and the development of hypoxia of hepatocytes and their necrosis;
- ethanol leads to the formation of free radical compounds, which causes significant violations of the structure and function of the cell membranes.

A wide range of drugs is used to correct the effects of alcoholism [11]. Most of them are pharmaceuticals, but recently researchers and specialists have begun to pay attention to medicines of natural origin – herbal medicines and natural remedies [12 - 17]. Among the latter - mineral waters (MW), which virtually have no contraindications, have a powerful biological, detoxifying, therapeutic effect [18 - 23]. MW affect the activity of liver enzymes, improving its functional state, and most importantly, have a stimulating effect on the reparative processes of this organ [24, 25, 26]. The positive effect of MW internal use is associated with the peculiarities of their physicochemical composition, osmolarity, the presence of biologically active substances, trace elements, etc. [27 - 35].

In this aspect, studies to determine the therapeutic properties of the MW with different chemical composition on the course of alcohol-induced pathologies in laboratory animals are appropriate and important, as they allow to obtain fairly rapid results, which greatly spread the idea of pathogenetic and sanogenetic mechanism of this suffering.

The aim of the study was to investigate the effect of internally use natrium chloride mineral water on the rat body with alcohol intoxication.

Methods

Experimental studies were performed on 34 white female self-breeding breed of the Wistar rats with a body weight of 180.0 - 200.0 g. The animals were kept in the vivarium with standard drinking and light conditions. Animals' studies have been conducted according to the existing guidelines and legal documents [36, 37, 38].

The animals were divided into three groups. Group 1 consisted of 10 intact animals, the data of which served as controls. Group 2 - 12 animals with alcohol intoxication (AI). Group 3 - 12 animals, which on the background of the development of AI received a course of MW.

Model AI (Group 2) was reproduced by daily administration into the rats esophagus, at 12 o'clock in the afternoon, 25% ethanol solution at a dose of 1.5% of body weight (at the rate of 4 g of 96% ethanol per 1 kg of body weight) for 30 days [39]. To correct blood pressure from the 15th day to the 30th day of the experiment, MW was injected once a day into the rats esophagus with a soft probe at a dose of 1% of the animal's body weight (in the evening, at about 16.00), taking into account the daily rats biorhythm.

After 30 days of the experiment, the rats were removed from the experiment by decapitation method under the ether anesthesia. Macro- and microscopic assessment of the liver tissues structural state and functional organization was performed by removing of 2 pieces of rats liver. The first piece was passed through the increasing concentration alcohols and poured into celloidin. Made histological sections were stained with hematoxylin-eosin. Microscopic studies of the liver structural changes were performed on the obtained sections. To determine the activity of succinate dehydrogenase (SDG) and lactate dehydrogenase (LDH) according to Lloyd's recipe, the second piece was frozen with dry carbon dioxide (-70 °C) and histochemical reactions were performed on prepared cryostat sections [40].

Metabolic studies were performed by biochemical methods. The activity of reamination enzymes - alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase and the content of total bilirubin and its fraction was determined in the blood serum. Creatinine and urea content were also determined. The state of the antioxidant system (AOS) in the serum was studied by catalase activity, and the state of the prooxidant system / lipid peroxidation (LPO) – by the content of malonic dialdehyde (MDA). A thymol test was also performed. The state of energy-dependent transmembrane ion transport was determined by the activity of Na + / K + -ATPase and Ca2 + / Mg2 + -ATPase in the liver homogenate.

Used methods were approved by the Ministry of Health of Ukraine [41]. All data were processed using the statistical package Statistica 10.0 (Statsoft / Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean ± standard error of the mean (SEM) or mean ± standard deviation. Significance was assessed using the one-way ANOVA followed by t-test. Values were considered statistically significant when P value was less than 0.05.

Rats of group 3 received a natural MW from underground well № 991-P "Sanatorium Resort Orlivshchyna", Ltd., Dnipropetrovsk region, Novomoskovsk district, the village of Orlivshchyna. This MW characterized as Natrium chloride water with average mineralization. Chemical composition of the MW has the following form: total mineralization 5.75 g / l, chloride ions 2900 mg / l, sulfate ions - 200 mg / l, bicarbonate ions - 270 mg / l, sodium and potassium ions - 1800 mg / l, calcium ions - 160 mg / l and magnesium ions - 75 mg / l.

Results

On the thirtieth day of pathological process the significant changes of metabolic parameters were found in group 2 rats (Table 1). Manifestations of increased permeability of hepatocyte membranes are an increase in the level of ALT and AST in the blood by 10% ($p < 0.01$) and 17% ($p < 0.01$), respectively. Against this background, the content of total blood protein decreases significantly by 11% ($p < 0.05$), which ultimately indicates the suppression of reamination and protein metabolism. The thymol test significantly increases by 38% and

the alkaline phosphatase activity increases more moderately by 23% (p <0.01), which confirms the presence of dystrophic processes in the liver parenchyma. The level of total bilirubin and its fractions did not differ from the indicators of group 1 of intact animals (p> 0.5). The development of AI is characterized by a significant imbalance in the AOC / POL system: against the background of inhibition of the activity of the enzyme antioxidant protection of catalase by 10% (p <0.01), there was an increase in prooxidant processes - an increase of 30% (p <0.01) MDA content. It should be emphasized a significant increase in creatinine by 20% and urea by 138%, which is evidence of violations of the processes of processing and excretion of toxins in endogenous detoxification systems. An imbalance in the system of energy-dependent transmembrane ion transport was determined - the activity of Mg2 + - Ca2 + - ATPase increased significantly by 30% (p <0.05) against the background of a decrease in the activity of Mg2 + Na + / K + -ATPase by 42% (p <0, 05). Deviations from the pigment metabolism were not detected, as evidenced by the lack of significant changes in the content of bilirubin and its fractions in comparison with the group 1 intact animals.

Macroscopic examination of the liver of rats of group 2 revealed that its surface is smooth, the anterior edge is sharp, the liver tissue is yellow-brown color.

On microscopic examination, the lobular structure of the liver was intact. The location of hepatocytes in the lobe is disordered. The cytoplasm of hepatocytes is granular,the nuclei had medium size, juicy. A significant number of hepatocytes have fatty inclusions. Between cells and in some of them - the inclusion of eosinophilic hyaline masses. The vessels are full-blooded. SDH activity in hepatocytes is (6.00 ± 0.37) c.u. of optical density; LDH activity - (6.00 ± 0.20) c.u. of optical density, the rate of LDH activity in hepatocytes - (4.00 ± 0.21) c.u. of optical density, i.e. increasing the use of alternative substrates for energy production.

In general, it can be argued that on the thirtieth day of alcohol intoxication, the manifestations of dystrophy in the liver are determined, which is accompanied by suppression of the processes of reamination and protein metabolism against the background of increasing manifestations of hypoxic nature; violation of the processes of processing and excretion of toxins in endogenous detoxification systems; reducing the activity of energy-dependent transmembrane transport processes.

In rats of group 3, which on the background of the development of AI received natrium chloride MW were found improvement in metabolic parameters (Table 1). Restoration of catalase activity (p> 0.5), alkaline phosphatase activity (p> 0.5), total protein content (p> 0.5) and creatinine (p> 0.5) was determined. This significantly reduces the urea content, but does not reach the group 1 level and differs significantly from it (p<0,01). The restoration of the level of thymol test (p> 0,5), positive changes in the activity of reamination enzymes - reduced activity of AST (p <0.01) and ALT (p <0.01) were detected. The MDA content and catalase activity reach the level of the control group, which indicates the restoration of balance in the AOS / POL system. There is a significant decrease in pigment metabolism - total, direct and indirect bilirubin (p <0.01), which indicates the activation of conjugation and bile excretion in the liver. Partial normalization of the activity of ATPase indicators and the energy supply system of ion transport was determined. The activity of the enzyme Mg2 + -Na + / K + -ATPase, although increasing, but remains below normal (p <0,01).

Morphological studies of epy group 3 rats liver found that the lobular organization of the parenchyma is preserved, the vessels are stagnant full-blooded. Hepatocytes are disordered. Their cytoplasm is basophilic homogeneous, the nuclei are small dark. SDG activity – in the center of the lobe (5.0 ± 0.11) c.u. of optical density, on the periphery – (6.0 ± 0.23) c.u. of optical density, LDH activity – (7.0 ± 0.30) c.u. of optical density generally returns to the control group.

Discussion

1. Under the influence of the course with natrium chloride MW determined reduction of fatty degeneration, improvement of structural and functional

organization of the liver parenchyma (lipid and hyaline inclusions disappear), which can be the basis for improving the detoxification function of the liver.

2. The positive influence of the MW on the metabolism state was determined: the processes of energy-dependent transmembrane transport and reamination, protein metabolism were restored; the reduction of hypoxic manifestations and the increase of the activity of endogenous detoxification and recovery processes of lipid peroxidation and antioxidant protection system have been established.

3. It can be assumed, that the established effects of the using natrium chloride MW indicates, that this MW has a protective effect on the structural and functional state of the liver and metabolism of the rats against the background of the development of alcohol intoxication.

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Table 1. The effect of sodium chloride MW on the metabolic parameters of rats with alcohol intoxication

Indexes	Group 1 ($M_1 \pm m_1$)	Group 2 ($M_2 \pm m_2$)	Group 3 ($M_3 \pm m_3$)
ALT, U/l	175,43 ± 1,65	193,93 ± 3,22*	186,05 ± 2,27*
AST, U/l	241,18 ± 3,51	282,89 ± 6,50*	268,57± 4,08*
De Ritis Ratio	1,38 ± 0,02	1,46 ± 0,05	1,44 ± 0,39
Total bilirubin, mmol/l	8,44 ± 0,28	7,93 ± 0,24	3,51 ± 0,15*
Bilirubin direct, mmol/l	3,06 ± 0,18	3,07 ± 0,22	1,26 ± 0,10
Bilirubin indirect, mmol/l	5,38 ± 0,15	4,86 ± 0,45	2,25 ± 0,16*
Thymol test, c.u.	1,06 ± 0,15	1,46 ± 0,10*	1,02 ± 0,12
Total protein, g/l	68,70± 2,74	61,33 ± 0,84*	69,40 ± 2,18
Creatinine, μmol/l	47,80± 0,63	57,43 ± 2,84	45,10 ± 1,74
Urea, mmol/l	2,80 ± 0,27	6,67 ± 0,21*	4,03 ± 0,22*
Mg2 + - Ca2 + -ATPase, mg P/g tissue	9,11 ± 0,93	11,75 ± 0,67*	8,11 ± 0,46
Mg2 + -Na + / K + -ATPase, mg P/g tissue	6,40 ± 0,62	3,72 ± 0,24*	4,28 ± 0,35
POL (MDA), nmol/(min×mg)	5,94 ± 0,21	7,79 ± 0,31*	6,09 ± 0,26
Catalase, %	76,70 ± 1,52	68,91 ± 2,14*	74,84 ± 1,61
Alkaline phosphatase, U/l	369,10±12,41	455,22±20,61*	409,34±19,21

Notes. ($M_1 \pm m_1$), ($M_2 \pm m_2$) and ($M_3 \pm m_3$) are arithmetic means with errors; *- $p < 0.05$